0022-3565/88/2473-1046$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 247, No. 3
Printed in U.S.A.

Pharmacology and Stereoselectivity of Structurally Novel Cannabinoids in Mice[1]

PATRICK J. LITTLE, DAVID R. COMPTON, M. ROSS JOHNSON,[2] LAWRENCE S. MELVIN[3] and BILLY R. MARTIN
Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia
Accepted for publication August 22, 1988

ABSTRACT

The pharmacological effects of three stereoisomeric pairs of structurally novel cannabinoids were tested after i.v. administration in mice for depression of spontaneous activity and the production of hypothermia, antinociception and catalepsy. The (−)-enantiomers were as much as 770 times more potent than Δ^9-6a,10a-*trans*-tetrahydrocannabinol and were 7 to 2000 times more potent than their respective (+)-enantiomers. The order of potency for cannabinoid-induced effects was spontaneous activity > antinociception > hypothermia ≥ catalepsy. Levonantradol was active between 0.123 to 1.5 mg/kg, whereas dextronantradol, its (+)-enantiomer was inactive. (−)-CP 55,244 and (−)-CP55,940 (see scheme 1) analogs which lack the dihydropyran ring were 5 to 775 times more potent than Δ^9-6a,10a-*trans*-tetrahydrocannabinol and 30 to 2000 times more potent than their respective (+)-enantiomers. Some separation of effects was demonstrated with (+)-CP 55,243 and (−)-CP 56,667 which were inactive in producing hypothermia and catalepsy but were active in the spontaneous activity and tail-flick procedures. The high degree of enantioselectivity and potency of these nonclassical cannaboinoids are indicative of a highly specific mechanism of action such as a receptor.

Δ^9-THC and other cannabinoids produce a wide spectrum of pharmacological effects in both laboratory animals and humans. These pharmacological properties include effects which are unique to Δ^9-THC and other psychoactive cannabinoids, such as production of static ataxia in the dog, and their discriminative stimulus properties. However, the cannabinoids also produce many effects which are shared by agents in other pharmacological classes, which include their analgesic, antiemetic, anticonvulsant and hypothermic effects. The mechanisms of action by which cannabinoids exert their pharmacological effects remain unclear (Martin, 1986). Cannabinoids may work through a general anesthetic-like membrane perturbation (Lawrence and Gill, 1975) thereby altering neuronal conduction or ion fluxes. There is also evidence which supports the opposite view that Δ^9-THC is working through a highly specific mechanism such as an interaction with a receptor. This view is supported by a well-defined SAR demonstrated with cannabinoids (Razdan, 1986), the demonstration of high affinity, saturable binding in the brain (Nye *et al.*, 1985) and stereoselectivity of pharmacological effects with the naturally occurring (−)-Δ^9-THC being 5 to 100 times more potent than the synthetic (+)-enantiomer (Dewey *et al.*, 1984). This is a modest degree of stereoselectivity; however, it is perplexing that the degree of stereoselectivity varies with the animal model chosen and even varies within the same species. If a specific receptor interaction was involved, one would expect a higher degree of stereoselectivity and less discrepancy between test systems.

The antinociceptive properties of the cannabinoids have been widely documented. It was generally concluded that Δ^9-THC when administered s.c. to mice and rats displayed weak analgesic activity in a variety of antinociceptive tests. 11-hydroxy-Δ^9-THC, an active metabolite of Δ^9-THC, was reported to have analgesic activity comparable to that of morphine when administered s.c. and this effect was naloxone reversible (Wilson and May, 1975). To determine if the analgesia demonstrated with Δ^9-THC was due to its metabolism to 11-hydroxy-Δ^9-THC analogs which could not be hydroxylated at C11 were synthesized, and it was demonstrated that (−)-9-nor-9β-hydroxyhexahydrocannabinol was approximately as potent as morphine (Wilson and May, 1975, Wilson *et al.*, 1976). These findings led to the synthesis and testing of many novel cannabinoid-based analogs for their potential therapeutic use as analgesics (Johnson *et al.*, 1981, Melvin and Johnson, 1987). Many of these compounds utilized the 9β-hydroxy-hexahydrocannabinol with structural changes in the side chain or in the benzopyran ring. Many potent analgesics were discovered and in addition very high degrees of enantioselectivity were demonstrated in all tests and species.

Received for publication April 25, 1988.
[1] This work was supported by National Institute on Drug Abuse Grant DA-03672 and National Institute on Drug Abuse Training Grants DA-07027 and DA-05330.
[2] Present address: Glaxo Inc., 5 Moore Dr., Research Triangle Park, NC 27709.
[3] Present address: Pfizer INc., Central Research, Groton, CT 06340.

ABBREVIATIONS: THC, 6a,10a-*trans*-tetrahydrocannabinol; SAR, structure-activity relationships; % MPE, percentage of maximum possible effects.

This investigation examined the pharmacology of three sets of enantiomers in mice. The pairs of enantiomers were chosen because they represent the most potent in the series of structural classes, as well as the most potent ring systems developed from the cannabinoid nucleus. The enantiomers were tested for their ability to reduce locomotor activity, produce hypothermia, analgesia and catalepsy. A battery of tests was chosen in order to determine if the enantiomers possessed a full spectrum of cannabimimetic effects or whether or not some separation of effects have been achieved by the structural modifications. The demonstration of a high degree of enantioselectivity in tests other than antinociception and of high potency in all tests would be characterisitic of a specific interaction such as those which are receptor mediated.

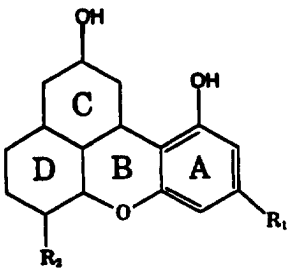
Methods

Materials. ICR male mice (Dominion Laboratories, Dublin, VA) weighing 24 to 30 g were used for all test procedures. Mice were maintained on a 12-hr light/dark cycle and had free access to food and water. Δ^9-THC was provided by the National Institute on Drug Abuse. Levonantradol, dextronantradol, CP-55,940, CP-56,667, CP-55,244 and CP-55,243 were obtained from Pfizer Pharmaceuticals (Groton, CT). To ease in the presentation and discussion of the results the following nomenclature will be utilized to refer to the pairs of enantiomers. CP 55,940 and CP 56,667 are bicyclic structures which will be referred to as (−)- and (+)-AC respectively. CP 55,244 and CP 55,243 are the (−)- and (+)-enantiomers of tricyclic compound referred to as ACD (scheme 1). This nomenclature has been adopted from Johnson and Melvin, 1986. All drugs were first dissolved in a 1:1 emulphor-ethanol solution, and diluted to the desired concentration with 0.9% saline to yield a final vehicle of 1:1:18 (emulphor-ethanol-saline). All drugs were administered i.v. in the tail vein with an injection volume of 0.1 ml/10 g of b.wt. Each mouse was then tested in all procedures as described below. All tests were performed at the time which Δ^9-THC elicited a peak response in each individual test.

Spontaneous activity. Mice were placed into individual photocell activity cages (11 inches × 6.5 inches) immediately after i.v. injection of the vehicle or cannabinoid. Mice were allowed to acclimate for 5 min and then interruptions of a single photocell beam were recorded for the next 10 min.

Hypothermia. Mice were acclimated in the laboratory (ambient temperature 21–24°C) overnight. Rectal temperatures were determined before drug or vehicle administration with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) and a thermistor probe. Rectal temperatures were again measured 60 min after administration of the drug or vehicle. The dose required to produce a 3°C decrease (approximately a half-maximal response) in rectal temperature was determined by linear regression analysis.

Analgesia. Tail-flick reaction time to a heat stimulus was determined after drug or vehicle administration using the method of D'Amour and Smith (1941) as modified by Dewey *et al.* (1970). Preinjection control values (2–4 sec) were determined for all animals. Mice were retested 20 min after i.v. administration of the drug or vehicle, and the latency to the tail flick response was recorded. A 10-sec maximum latency was set to prevent tissue damage. Data were recorded as change in latency between pre- and postinjection testing for each animal. Data were expressed as % MPE, where % MPE was determined by the following method: [(test latency − control latency) ÷ (10 sec − test latency)] ×100.



Scheme 1. Nomenclature for the ring systems for the cannabinoids.

Catalepsy. Catalepsy was determined by using a slight modification of the ring test as developed by Pertwee (1972). Mice were injected i.v. with either vehicle or cannabinoid and 1.5 hr after treatment were placed on a ring (5.5 cm in diameter) which was attached to a ring stand and raised to a height of 16 cm. Mice were rated for catalepsy by observers who were blind with regard to treatment. The amount of time (seconds) in a 5-min test session in which the mouse was motionless (except for respiratory movements) was determined. Mice which either fell or actively jumped from the ring were allowed 5 such "escapes." If these occurred before 2.5 min the animal was disregarded. An immobility index was determined by dividing the amount of time spent motionless by the length of the test session (usually 300 sec) and multiplying by 100 to determine an immobility index for each mouse.

Antagonism studies. Mice were pretreated i.v. with a dose of an analog which had little or no pharmacological effects on its own. Ten minutes later 6 mg/kg of Δ^9-THC was administered i.v. and mice were tested in the above procedures.

Statistical analysis. ED_{50} values with 95% CL were determined for reduction in locomotor activity, for the production of analgesia using the %MPE, and for the production of catalepsy using the immobility index by the method of Litchfield and Wilcoxon (1949). Statistical differences between vehicle and drug treatment were determined by the Dunnett's *t* test. An analysis of variance with a Scheffe post hoc test was utilized to determine if there were statistical differences between treatment groups in the antagonism studies.

Results

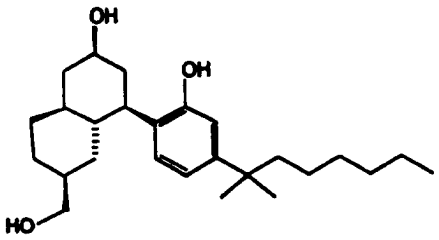
The (−)-enantiomers of all of the compounds were very potent in all the tests. The order of potency in all tests (−)-ACD > (−)-AC > levonantradol. In addition to demonstrating the same rank order potency in all tests, the (−)-enantiomers also displayed similar relative potencies in all the tests. The order of potency (from lowest ED_{50} value to highest) was spontaneous activity > tail-flick > hypothermia ≥ catalepsy, where the dose required to lower rectal temperature by 3°C was used as the criteria for the hypothermic response.

(−)-ACD decreased spontaneous activity, lowered rectal temperature, produced analgesia and catalepsy in a dose responsive manner from 0.3 to 300 μg/kg (table 1). At a dose of 1 mg/kg the antinociceptive response was decreased dramatically but this appeared to be the result of extreme hyperexcitability seen in some mice. (−)-ACD was the most potent enantiomer tested, with ED_{50} values ranging from 4 to 85 μg/kg depending upon the behavioral measure (table 4). This enantiomer was 100 to 500 times more potent than Δ^9-THC in the mouse. In addition to its extremely high potency, there was a very high degree of enantioselectivity, the (+)-enantiomer was inactive at doses up to 10 mg/kg in the tail-flick and catalepsy procedures and failed to lower rectal temperature in a dose-responsive manner. (+)-ACD did decrease locomotor activity in a dose-responsive manner and had an ED_{50} of 8 mg/kg (table 4) which was 2000 times greater than the ED_{50} for the (−)-enantiomer. (−)-ACD was at least 1000, 200 and 118 times as potent as (+)-ACD in the tail-flick, hypothermia and catalepsy procedures, respectively.

(−)-AC was another potent cannabinoid which was active in all tests in a dose-responsive manner between 10 to 1000 μg/kg (table 2). The (+)-enantiomer of this analog was also active in decreasing locomotor activity between 1 to 10 mg/kg (ED_{50},

TABLE 1

Agonist effects of CP 55,244 and CP 55,243 in mice

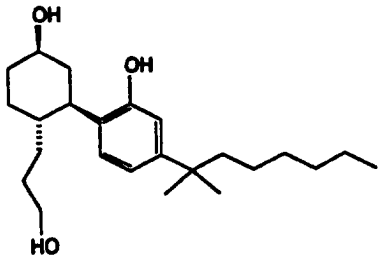


Dose	n	Spontaneous Activity[a]	Temperature Change (°C)[b]	Tail-Flick[c]	Immobility Index[d]
		(−)-ACD (CP 55,244)			
0.3 μg/kg	6	129	−0.7	18	3
1 μg/kg	6	112	+0.1	13	2
3 μg/kg	18	43	0.0	44	23
10 μg/kg	12	8	−0.5	74	28
30 μg/kg	12	21	−1.4	64	35
100 μg/kg	6	2	−5.8	100	55
300 μg/kg	6	0	−6.5	89	77
1 mg/kg	6	0	−5.7	33	67
ED_{50} (95% CL) μg/kg		4 (0.8–17)	50[e]	10 (0.3–298)	85 (30–243)
		(+)-ACD (CP 55,243)			
1 mg/kg	24	105	+0.2	9	13
3 mg/kg	24	85	+0.6	22	21
10 mg/kg	24	40	−0.6	30	22
ED_{50} (95% CL) mg/kg		8 (5–13)			

[a] Spontaneous Activity values represent the percentage of activity of vehicle-treated mice using interruptions of a photocell beam in a 10-min session, 5 min after i.v. injection.
[b] Values represent the change in rectal temperature as measured before drug treatment and redetermined 60 min after i.v. administration.
[c] Values represent the % MPE (10 sec cutoff) in the tail-flick procedure tested 20 min after i.v. administration.
[d] Values represent the percentage of time that mice remained immobile on the ring in a 5-min test session 90 min after i.v. administration.
[e] Value represents dose required to decrease rectal temperature by 3°C.

TABLE 2

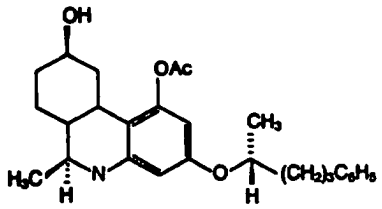
Agonist effects of CP 55,940 and CP 56,667 in mice



Dose	n	Spontaneous Activity[a]	Temperature Change (°C)[b]	Tail-Flick[c]	Immobility Index[d]
		(−)-AC (CP 55,940)			
10 μg/kg	6	81	+0.1	20	6
30 μg/kg	12	57	+0.2	43	17
100 μg/kg	18	32	−2.2	63	29
300 μg/kg	12	5	−2.8	55	52
1 mg/kg	6	8	−4.4	77	64
ED_{50} (95% CL) μg/kg		40 (17–92)	349[e]	87 (25–301)	347 (134–897)
		(+)-AC (CP 56,667)			
1 mg/kg	12	137	+0.4	9	10
3 mg/kg	18	43	0.0	28	19
6 mg/kg	6	19	−0.3	48	6
10 mg/kg	18	17	−1.3	69	36
ED_{50} (95% CL) mg/kg		3 (0.6–15)		6 (3–10)	

[a–e] See footnotes in table 1.

TABLE 3
Agonist effects of CP 50,556-1 and CP 53,870-1 in mice



Dose	n	Spontaneous Activity[a]	Temperature Change (°C)[b]	Tail-Flick[a]	Immobility Index[a]
		Levonantradol (CP 50,556-1)			
10 μg/kg	6	163	+2.1	12	21
30 μg/kg	6	241	+1.2	2	5
100 μg/kg	12	35	+0.3	65	15
300 μg/kg	18	34	−2.0	71	34
1 mg/kg	18	6	−3.5	61	39
3 mg/kg	12	1	−5.3	77	69
ED_{50} (95% CL) mg/kg		0.123 (0.007–2.0)	0.769[a]	0.287 (0.065–1.3)	1.5 (0.4–5.9)
		Dextronantradol (CP 53,870-1)			
1 mg/kg	18	130	+0.2	9	14
3 mg/kg	18	126	0.0	13	18
10 mg/kg	18	86	−0.1	20	10

[a–c] See footnotes in table 1.

TABLE 4
Summary of the pharmacological effects of the nonclassical cannabinoids
Values represent the ED_{50} with 95% confidence levels in parentheses and are expressed as milligrams per kilogram.

	Levonantradol	Dextronantradol	Stereoselectivity[a]	Potency[b]
Spontaneous activity	0.123 (0.007–2.0)	Not active[c]	>81	25
Tail flick	0.287 (0.065–1.3)	Not active	>35	4
Catalepsy	1.5 (0.4–5.9)	Not active	>7	1
Rectal temperature[d]	0.769	Not active	>13	31
	(−)-AC	(+)-AC		
Spontaneous activity	0.040 (0.017–0.092)	3 (0.6–15)	75	77
Tail flick	0.087 (0.025–0.301)	6 (3–10)	69	15
Catalepsy	0.347 (0.134–0.897)	Not active	>29	5
Rectal temperature	0.349	Not active	>29	69
	(−)-ACD	(+)-ACD		
Spontaneous activity	0.004 (0.0008–0.017)	8 (5–13)	2000	773
Tail flick	0.010 (0.0003–0.298)	Not active	>1000	126
Catalepsy	0.085 (0.03–0.243)	Not active	>118	19
Rectal temperature	0.050	Not active	>200	480
	(−)-Δ⁹-THC			
Spontaneous activity	3.1 (1.2–8.2)			1
Tail flick	1.3 (0.6–2.5)			1
Catalepsy	1.6 (0.9–2.7)			1
Rectal temperature	24			1

[a] Stereoselectivity reflects the relative potencies of the isomeric pairs by comparing ED_{50} values.
[b] Potency to that Δ⁹-THC.
[c] Not active refers to the fact that an ED_{50} was not obtained at doses up to 10 mg/kg.
[d] Values for rectal temperature represent the dose needed to decrease temperature by 3°C.

3 mg/kg) which is similar to (+)-ACD. In addition, (+)-AC was active in the tail-flick assay within this same dose range with an ED_{50} of 6 mg/kg (table 2). (−)-AC was approximately 10 to 50 times as potent as Δ⁹-THC with ED_{50} values ranging from 40 to 349 μg/kg (table 4). The (−)-enantiomer was 75 and 69 times more potent than the (+)-enantiomer in decreasing locomotor activity and in producing analgesia, respectively. (+)-AC was not active in producing hypothermia and catalepsy, thus the (−)-enantiomer was at least 29 times as active as the (+)-enantiomers in these paradigms (table 4).

Levonantradol was the least potent of the (−)-enantiomers with ED_{50} values ranging from 0.123 to 1.5 mg/kg (table 4); however, levonantradol was found to be equipotent to 30 times as potent as Δ⁹-THC. Levonantradol was tested at doses between 0.01 to 3 mg/kg; however, it as not until 0.1 mg/kg that significant effects were seen. Dextronantradol was inactive in all tests up to 10 mg/kg (table 3). Levonantradol was at least 7 to 81 times more potent than dextronantradol.

The shape of the dose-response curves of the (−)-enantiomers of these nonclassical cannabinoids and Δ⁹-THC in mice

TABLE 5

Effects of pretreatment with (+)-enantiomers on Δ⁹-THC effects in mice

Compound	Test	Change in Δ⁹-THC Response[a]
Dextronantradol (3 mg/kg)	Spontaneous activity	+66
	Rectal temperature	+61
	Tail-flick	+27
	Catalepsy	−3
(+)-ACD (3 mg/kg)	Spontaneous activity	−11
	Rectal temperature	+7
	Tail-flick	−16
	Catalepsy	+14
(+)-AC (1 mg/kg)	Spontaneous activity	−35
	Rectal temperature	+20
	Tail-flick	−10
	Catalepsy	+39

[a] Values represent the percentage of change of the effects of Δ⁹-THC after pretreatment with the respective (+)-enantiomers as compared to pretreatment with the vehicle followed by Δ⁹-THC. +, an enhancement in the effects of Δ⁹-THC; −, a dimunition in the effects of Δ⁹-THC.

were very similar with the exception of catalepsy production. The dose-response curve for catalepsy for Δ⁹-THC (data not shown) reached a plateau between 6 to 30 mg/kg with immobility indices ranging from 42 to 58%; however, there were no statistically significant differences between the degree of catalepsy in this range. In contrast, no plateau was seen with any of the (−)-enantiomers of the nonclassical cannabinoids. The immobility index of levonantradol rose dramatically between 1 and 3 mg/kg from 35 to 68%, whereas the dose-response curve for (−)-AC rose steadily throughout the entire range from 10 μg to 1 mg/kg in which a maximum value of 64% was reached (table 4). Higher doses of (−)-AC could not be tested due to the fact that at 1 mg/kg the mice were hyperexcitable, ataxic and displayed severe clonus. (−)-ACD produced catalepsy in a smooth, dose-responsive manner between 1 and 300 μg/kg producing a maximum immobility index of 77% at 300 μg/kg. There was a slight but nonsignificant decrease in catalepsy at 1 mg/kg in which an increase in the ataxia and hyperexictability was also seen.

The ability of the (+)-enantiomers of these cannabinoid analogs to attenuate the effects of Δ⁹-THC in the mouse are shown in table 5. Doses which had little or no effect of their own were chosen based on the dose-effect curves generated for the (+)-enantiomers. A dose of 6 mg/kg of Δ⁹-THC was used as this dose produces robust effects in all tests, but did not produce a maximal response. There was no consistent attenuation of the effects of Δ⁹-THC by any of the (+)-enantiomers, in contrast there were instances in which pretreatment with the (+)-enantiomers enhanced the effects of Δ⁹-THC. This phenomenon was best demonstrated with 3 mg/kg of dextronantradol in which the suppression of locomotor activity and rectal temperature was enhanced greatly and the antinoceptive response was also increased markedly.

Discussion

The pharmacological profiles of these structurally nonclassical cannabinoids in mice sheds more light on both the structural requirements for the cannabinoid behavioral effects as well as possible mechanisms of action. As far as the SAR is concerned the potency of the (−)-enantiomers of ACD and AC demonstrates that structural changes concerning areas of the cannabinoid nucleus unexamined previously may be important in maximizing the pharmacological effects of cannabinoids. (−)-ACD is a tricyclic analog which lacks the dihydropyran ring; however, it retains the phenolic and alcoholic hydroxyls and an aliphatic side chain. These three functional constituents appear necessary for activity in a variety of antinociceptive models (Johnson and Melvin, 1986; Melvin and Johnson, 1987) as well as cannabimimetic activity in other behavioral paradigms such as dog static-ataxia and overt behavior in the rhesus monkey (Razdan, 1986). (−)-ACD retained activity despite the lack of the dihydropyran ring and the addition of a hydroxymethyl in a postion that has not been fully explored in relationship to the cannabinoids.

Further evidence to suggest that this area may be important for cannabimimetic activity was the potency of (−)-AC, a bicyclic cyclohexanol with a propyl hydroxy chain extending from the C ring. It has been demonstrated that the length of this alkyl chain is important for retaining the antinociceptive properties of other AC analogs. Maximal activity in the phenylbenzoquinone stretching test is obtained when the alkyl group is either a propyl or butyl, and activity is lost when the alkyl chain exceeds 4 carbons. In addition, placement of a hydroxyl group on the last carbon of the alkyl chain increases the potency approximately 2-fold (Melvin and Johnson, 1987). This suggests that this area of the cannabinoid may also have a strict SAR. The hydroxyl group in this compound approximates the position of the hydroxyl group in (−)-ACD. (−)-AC was 4 to 10 times less potent than (−)-ACD which is in agreement with its activity in a number of antinociceptive tests (Johnson and Melvin, 1986). The degree of enantioselectivity of these bicyclic enantiomers was less than that demonstrated with the enantiomers of the tricyclic ACD. The reason for the decreased potency and enantioselectivity of the AC enantiomers compared to the ACD enantiomers could be that the propyl hydroxy moiety in ACD is held rigid by the rest of the ring structure, whereas this would not be the case with the AC enantiomers.

Levonantradol, which retains the traditional ABC tricyclic nucleus of Δ⁹-THC was the least potent of the (−)-enantiomers tested. Levonantradol which differs from Δ⁹-THC in that the pyran oxygen is replaced by a weakly basic ntirogen and the alkyl side chain is replaced by an aryloxy side chain. Levonantradol was equipotent to Δ⁹-THC in producing catalepsy, but was 25 and 31 times more potent in decreasing spontaneous activity and rectal temperature, respectively. These latter ratios are similar to the relative potency of levonantradol in the dog static-ataxia model in which levonantradol was approximately 20 times more potent than Δ⁹-THC (Mast *et al.*, 1979) and in a number of antinociceptive tests in which levonantradol was 20 to 100 times more potent (Milne *et al.*, 1980). This suggests that the optimal structure for cannabimimetic activity may not lie with the traditional tricyclic nucleus.

The pharmacological profiles and the shape of the dose-response curves of the (−)-enantiomers of these nonclassical cannabinoids and of Δ⁹-THC in mice were very similar suggesting a similarity in the mechanisms of action between these nonclassical cannabinoids and Δ⁹-THC. The mechanisms of action by which cannabinoids exert their behavioral effects are unknown and may represent a composite of both specific mechanisms of action as well as nonspecific membrane effects. The 20-fold difference in ED_{50} value for (−)-ACD in the different tests suggests that the effects produced by cannabinoids may be mediated by multiple sites and/or mechanisms of action.

It has been postulated that alterations in adenylate cyclase

activity may be involved in mediating some of the pharmacological effects of cannabinoids. Cannabinoids have been shown to alter adenylate cyclase activity in a variety of tests systems (Martin, 1986); however, there is disagreement on the precise effects of Δ^9-THC on adenylate cyclase. In neuroblastoma cells, Δ^9-THC and levonantradol have been shown to inhibit adenylate cyclase in the nanomolar range, with levonantradol being approximately 10 times more potent (Howlett, 1985). Additionally, enantioselectivity for the inhibition of adenylate cyclase was demonstrated in that dextronantradol was a very poor inhibitor of this enzyme (Howlett, 1987). A direct coupling to a receptor operated adenylate cyclase system involved has been suggested by the fact that the inhibitory G protein, G_i may be involved, based on the fact that pertussis toxin (which ADP-ribosylates G_i) abolished the inhibitory effects of Δ^9-THC on adenylate cyclase (Howlett *et al.*, 1986). Recently, it has been demonstrated that cannabinoids which are potent inhibitors of adenylate cyclase *in vitro* have potent analgetic effects *in vivo* (Howlett *et al.*, 1988) suggesting that the antinociceptive effect of the cannabinoids may be mediated through an inhibition of adenylate cyclase. It remains unclear whether other pharmacological effects of the cannaboids can be associated with alterations in adenylate cyclase.

The degree of enantioselectivity demonstrated with these structurally novel cannabinoids, especially that of the tricyclic ACD compound, greatly exceeded that which has been demonstrated previously for either Δ^9-THC or Δ^9-THC (Dewey *et al.*, 1984) and is on the order of that demonstrated for the stereoisomers of morphine on a number of assays (Jacquet *et al.*, 1977). The enantioselectivity as well as the extreme potency of the (−)-enantiomers are certainly consistent with the existence of a highly specific site of action (*i.e.*, a receptor) for the cannabinoids.

Acknowledgment

The authors wish to acknowledge the expert technical assistance of Mrs. Ramona L. Winckler.

References

D'Amour, F. E. and Smith, D. L.: A method for determining loss of pain sensation. J Pharmacol. Exp. Ther. **72**: 74–79, 1941.

Dewey, W. L., Harris, L. S., Howes, J. F. and Nuite, J. A.: The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone test. J. Pharmacol. Exp. Ther. **175**: 435–442, 1970.

Dewey, W. L., Martin, B. R. and May, E. L.: Cannabinoid stereoisomers: Pharmacological effects. *In* Handbook of Stereoisomers: Drugs in Pyschopharmacology, ed. by D. F. Smith, pp. 317–326, CRC Press, Boca Raton, FL, 1984.

Howlett, A. C.: Cannabinoid inhibition of adenylate cyclase biochemistry of the response in neuroblastoma cell membranes. Mol. Pharmacol. **27**: 429–436, 1985.

Howlett, A. C.: Regulation of adenylate cyclase in a cultured neuronal cell line by marijuana constituents, metabolites of delta-9-tetrahydrocannabinol, and synthetic analogs having psychoactivity. NIDA Res. Monograph Ser. **79**: 148–157, 1987.

Howlett, A. C., Johnson, M. R., Melvin, L. S. and Milne, G. M.: Nonclassical cannabinoid analgetics inhibit adenylate cyclase: Development of a cannabinoid receptor model. Mol. Pharmacol. **33**: 297–302, 1988.

Howlett, A. C., Qualy, J. M. and Khachatrian, L. L.: Involvement of G_i in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol. Pharmacol. **29**: 307–313, 1986.

Jacquet, Y. F., Klee, W. A., Rice, K. C., Iijima, I. and Minamikawa, J.: Stereospecific and nonstereospecific effects of (+)- and (−)-morphine: Evidence for a new class of receptors? Science (Wash. DC) **198**: 842–845, 1977.

Johnson, M. R. and Melvin, L. S.: The discovery of nonclassical cannabinoid analgetics. *In* Cannabinoids as Therapeutic Agents, ed. by R. Mechoulam, pp. 121–145, CRC Press, Boca Raton, FL, 1986.

Johnson, M. R., Melvin, L. S., Althius, T. H., Bindra, J. S., Harbert, C. A., Milne, G. M. and Weissman, A.: Selective and potent analgetics derived from cannabinoids. J. Clin. Pharmacol. **21**: 271S–282S, 1981.

Lawrence, D. K. and Gill, E. W.: The effects of Δ^1-tetrahydrocannabinol and other cannabinoids on spin-labeled liposomes and their relationship to mechanisms of general anesthesia. Mol. Pharmacol. **11**: 595–602, 1975.

Litchfield, L. T. and Wilcoxon, F. A.: A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. **96**: 99–113, 1949.

Martin, B. R.: Cellular effects of cannabinoids. Pharmacol. Rev. **38**: 45–74, 1986.

Mast, R. W., McIlhenny, H. M., Milne, G. M. and Johnson, M. R.: Behavioral effects and pharmacokinetics of nantradol hydrochloride, a potent, cannabinoid-related analgesic. Pharmacology (Basel) **21**: 270, 1979.

Melvin, L. S. and Johnson, M. R.: Structure-activity relationships of tricyclic and nonclassical bicyclic cannabinoids. NIDA Res. Monograph Ser. **79**: 31–47, 1987.

Milne, G. M., Koe, B. K. and Johnson, M. R.; Stereospecific and potent analgetic activity for nantradol-a structurally novel, cannabinoid-related analgetic. NIDA Res. Monograph Ser. **27**: 84–92, 1980.

Nye, J. S., Seltzman, H. H., Pitt, C. G. and Snyder, S.H.: High affinity cannabinoid binding sites in brain membranes labeled with [^{3}H]-5′-trimethylammonium Δ^8-tetrahydrocannabinol. J. Pharmacol. Exp. Ther. **234**: 784–791, 1985.

Pertwee, R. G.: The ring test: A quantitative method for assessing the "cataleptic" effect of cannabis in mice. Br. J. Pharmacol. **46**: 753–763, 1972.

Razdan, R. K.: Structure-activity relationships in cannabinoids. Pharmacol. Rev. **38**: 75–149, 1986.

Wilson, R. S. and May, E. L.: Analgesic properties of the tetrahydrocannabinols, their metabolites, and analogs. J. Med. Chem. **18**: 700–703, 1975.

Wilson, R. S., May, E. L., Martin, B. R. and Dewey, W. L.: 9-nor-9β-hdyroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties and comparison with tetrahydrocannabinols. J. Med. Chem. **19**: 1165–1167, 1976.

Send reprint requests to: Dr. Billy R. Martin, Department of Pharmacology and Toxicology, Box 613, MCV Station, Medical College of Virginia, Richmond, VA 23298.